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Effects of the specific phosphodiesterase inhibitors on alloxan-induced diabetic rabbit cavernous tissue *in vitro*

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An experimental study was done to examine a potential role of phosphodiesterase (PDE) inhibitors in the treatment of diabetic erectile dysfunction. Relaxant effect of specific PDE inhibitors were measured in strips of corpus cavernosum smooth muscle taken from control and diabetic groups. Diabetes mellitus was induced in New Zealand white rabbits using alloxan. Penises excised from diabetic rabbits 8 weeks after the induction of diabetes mellitus. In the organ bath strips from control and diabetic rabbit corpus cavernosum were precontracted and increasing doses of several PDE inhibitors were added. In the precontracted rabbit cavernous tissue, sulmazole and zaprinast specific PDE V inhibitors were equally potent and efficacious *in vitro* but amrinone, a specific PDE III inhibitor, exhibits low relaxant effects. All PDE inhibitors tested showed a similar relaxation effect on corpus cavernosum smooth muscle from control and 8-week diabetic rabbits. The present study provides the possibility of using selective PDE III and V inhibitors in the treatment of diabetic impotence. *International Journal of Impotence Research* (2001) **13,** 24–30.

Keywords: penile erection; phosphodiesterase inhibitors; diabetes mellitus

Introduction

Penile erection is a hemodynamic process involving relaxation of smooth muscle of the corpus cavernosum. This relaxation process results in an increased flow of blood into the trabecular spaces of the corpora cavernosa.[1,2] Although the control of cavernosal smooth muscle tone is complex, it is clear that the most important chemical mediator of cavernosal relaxation is nitric oxide (NO), released directly from nonadrenergic noncholinergic nerve endings and from the endothelium. Within the smooth muscle cell, NO stimulates the enzyme guanylate cyclase to convert guanosine triphosphate (GTP) into the active second messenger cyclic guanosine monophosphate (cGMP).[3,4] A number of recent studies have suggested that the cyclic nucleotides cAMP and cGMP are important second messengers in mediating the relaxation of various smooth muscle cells, including cavernous smooth muscle.[5,6] Phosphodiesterases (PDEs) are enzymes responsible for the hydrolysis of cGMP and cAMP to monophosphates. Of the PDE isozyme families PDE V and PDE VI are specific for cGMP as a substarate. PDE III and PDE IV are specific for cAMP, and PDE I and PDE II hydrolyse both cGMP and cAMP.[7] Recently, experimental studies showed PDE III and V isoenzymes to play an important role in the smooth muscle tone regulation of the corpus cavernosum.[8,9] Therefore, drugs that inhibit PDE potentiate the action of cGMP and thus facilitate penile erectile activity. This proposal is supported by reports that various more specific PDE inhibitors enhanced the NO-induced relaxation of human and rabbit corpus cavernosal tissue *in vitro*.[3,4]

Diabetes mellitus is a major risk factor for erectile dysfunction in both patients and animal models.[10,11] The pathophysiology of erectile dysfunction in diabetes is multifactorial, and the roles of neuropathy and arteriopathy are well recognised.[12] There is increasing evidence that diabetes leads to abnormal endothelial function and decreases nitric oxide synthase (NOS) activity in the penis, and it is conceivable that this impairment can lead to erectile dysfunction.[13]

Despite the fact that papaverine, a nonspecific PDE inhibitor has gained widespread acceptance in intracavernous injection therapy of diabetic erectile impotence, the role of PDE hydrolysis of cyclic nucleotides in regulating diabetic cavernosal smooth muscle tone needs further study. We therefore, examined the effects of various more specific PDE inhibitors on isolated diabetic rabbit corpus cavernosum tissue.

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Materials and methods

Sexually mature male New Zealand white rabbits weighing 2.0–2.5 kg were randomly assigned to control ($n=9$) and diabetic ($n=9$) groups. Diabetes was induced in the animals by injection with alloxan (150 mg/kg of body weight) via the ear vein following anesthesia using xylazine (5 mg/kg) and ketamine (20 mg/kg, i.m.). Rabbits received 10% glucose in the drinking water for the first 12 h, then fed regular rabbit chow and tap water for 8 weeks. The blood glucose concentration was measured by the glucose oxidase method. At the end of the first week, rabbits who demonstrated blood glucose levels of 250 mg/dl or above were accepted as diabetic.

Corpus cavernosum tissue

At the time of the study, rabbits were killed and the entire penis was removed. A ventral incision was made on the right and left corpora the tunica was dissected and the corpus cavernosum tissue was exposed and immediately placed in organ chambers. Each rabbit provided four strips of corpus cavernosum smooth muscle that were studied separately.

Organ chamber experiments

Strips of corpus cavernosum tissue measuring approximately $2\times2\times15$ mm were studied in 20 ml water jacketed tissue baths containing physiological salt solution for isometric tension measurement. The strips were tied with silk to a force transducer (Grass FT 03, Quincy, MA) at one end and fixed with silk ties to a glass support at the other end. The transducer output was recorded on a Grass polygraph model 79E. The solution was gassed with 95% O_2 and 5% CO_2 during the study and the temperature was maintained at 37°C by a thermoregulated water circuit. Resting load was set at 2 g a value which was previously found to be optimal for measurement of changes in tension of rabbit corpus cavernosal preparation. The preparations were allowed to equilibrate in Kreb's-bicarbonate for 1 h and during this time Kreb's-bicarbonate was replaced every 15 min with fresh solution. After equilibration the strips were contracted with phenylephrine ($10^{-6}-3\times10^{-6}$ M). These concentrations produced 70–80% of the maximal response to phenylephrine. After the phenylephrine-induced contraction had reached a plateau level, the concentration–response relationships for papaverine, a nonspecific PDE inhibitor, ($10^{-6}-3\times10^{-4}$ M), sulmazole, a specific PDE V inhibitor, ($10^{-6}-10^{-4}$ M), zaprinast, a specific PDE V inhibitor, ($10^{-6}-10^{-4}$ M) or amrinone, a specific PDE III inhibitor, ($10^{-6}-10^{-4}$ M), were obtained by adding one of those agents to the bath in a cumulative manner. The tissues were washed for 15 minutes before adding vehicle. In each strip, only one agonist was tested.

Analysis of data and statistics

Experimental values expressed as the mean ± s.e.m. Relaxant effects of agonists were expressed as a percentage of the precontraction to phenylephrine. In order to evaluate the effects of agonists; maximum responses (E_m) and pD_2 values (apparent agonist affinity constants; $-\log ED_{50}$) were calculated. The concentration–response data obtained in each individual experiment were plotted as the response/concentration (y) against the response (x). This produced a straight line relationship in each experiment as predicted from the Scatchard equation for drug receptor interaction.

Statistical comparisons between groups were performed using unpaired Student's t-test. Probabilities of less than 5% ($P<0.05$) were considered significant.

Solution and drugs

The ionic composition of the Kreb's solution was as follows (mM). NaCl118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, $MgSO_4$ 1.2, $KH_2PO_4$1.2, glucose 11. Fresh solutions were prepared on the day of the experiments. Phenylephrine hydrochloride, amrinone, sulmazole, zaprinast, papaverine hydrochloride and alloxan monohydrate were purchased from Sigma Chemical, St. Louis, MO, USA. Papaverine was dissolved in distilled water but amrinone, sulmazole and zaprinast dissolved in dimethylsulphoxide (DMSO). All drugs were prepared daily.

Results

The initial body weight was similar in the control and diabetic animals (Table 1). The body weights of diabetic rabbits 8 weeks after alloxan treatment were significantly less than those of control animals ($P<0.05$) (Table 1). At 8 weeks, the average blood glucose level was significantly greater in the diabetic group compared to the control group ($P<0.05$) (Table 1).

When tissue contraction was induced with 10^{-6} M -3×10^{-6} M phenylephrine for the study of responses to vasodilator agents, contractile tone

Table 1 Body weight and plasma glucose level in control group and diabetic group

	Body weight (kg)		*Plasma glucose (mg/dl)*
	Initial	*At 8 weeks*	*At 8 weeks*
Control ($n=9$)	2.2 ± 0.07	3.0 ± 0.16	98 ± 19
Diabetic ($n=9$)	2.3 ± 0.05	2.5 ± 0.15*	410 ± 87*

*$P<0.05$ statistically different from corresponding controls.
n = number of animals.

was similar in the diabetic and the control group. Tension induced by phenylephrine was 1608.6 ± 60.5 and 1664.9 ± 78.2 mg tension (mean $\pm$ s.e. $n=9$) in the diabetic and control groups, respectively.

Papaverine, zaprinast, sulmazole and amrinone produced concentration-dependent relaxation in precontracted corpus cavernosum strips obtained from controls and diabetic rabbits (Figures 1–4). In the precontracted cavernous tissue, zaprinast and sulmazole were equally potent and efficacious *in vitro* (Figures 2 and 3). Amrinone exhibited statistically lower relaxant effects than papaverine zaprinast or sulmazole with no change in the pD_2 value ($P<0.05$) see Figure 4 and Table 2.

The relaxation elicited by papaverine, zaprinast, sulmazole and amrinone was similar in diabetic and control groups and there were no significant changes in the pD_2 values see Figures 1–4 and Table 2.

The solvent used to dissolve amrinone, zaprinast or sulmazole (DMSO), at the volumes injected in the bath, was without apparent effect on either the relaxation or contractile responses of the corporal tissues (data not shown).

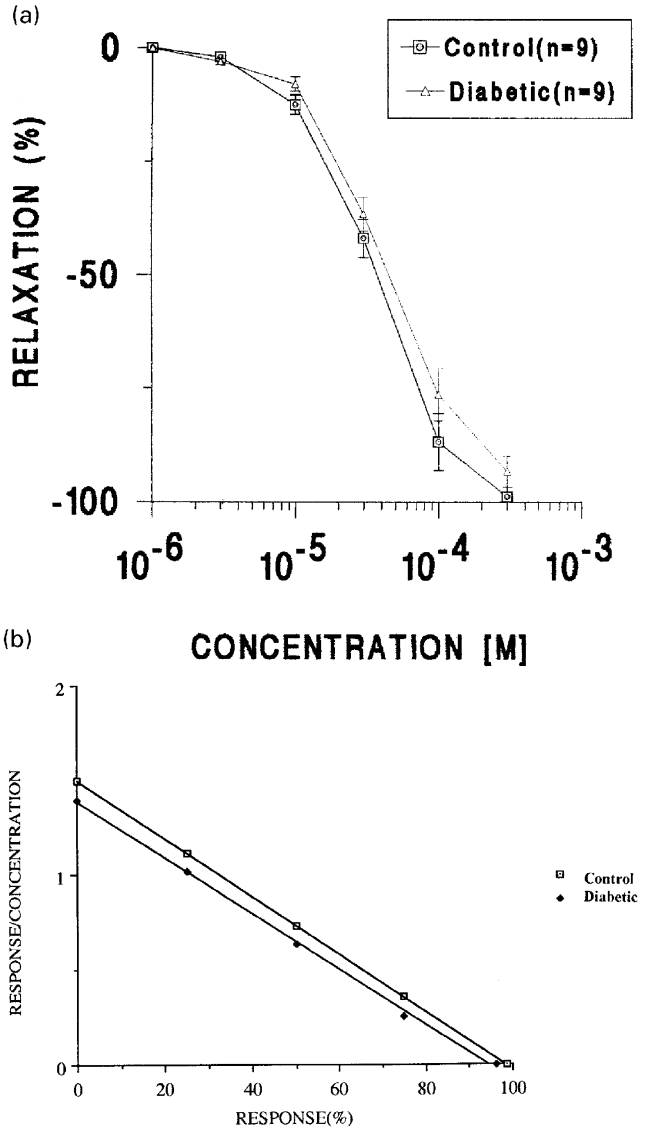


Figure 1 (a) Papaverine concentration–response curves in isolated rabbit corpus cavernosum strips precontracted with phenylephrine $10^{-6}-3\times10^{-6}$M. Each point is expressed as a percentage of the contraction induced by phenylephrine and is given as the mean $\pm$ s.e.m. Numbers in parentheses indicate the number of preparations used. (b) Scatchard analysis. E_m% and pD_2 values shown in Table 2. ($r=0.91$ for controls and $r=0.89$ for diabetic animals).

Discussion

The results presented in this study demonstrate that specific PDE III and V inhibitor relaxed the corpus cavernosum smooth muscle from control and 8-week diabetic rabbits. Alloxan is frequently used to cause experimental diabetes in animals. In this study, alloxan-induced diabetes produced a significant decrease in rabbit body weight and an increase in serum glucose concentration. It has been established that a relatively brief period of alloxan-induced diabetes can induce changes in the physiological mechanisms that modulate trabecular smooth muscle in rabbits.[10,11] The similarity of these changes to those in tissues from diabetic impotent men make the alloxan-induced diabetic rabbit a suitable model to investigate the effects of diabetes on penile smooth muscle.

The effects of many hormones and neurotransmitters are mediated through specific receptors coupled to adenylate cyclase and guanylate cyclase.[14–17] Phosphodiesterases inactivate cyclic nucleotides, and the three different PDE isozymes in human erectile tissue, PDE III, IV and V have been characterized.[8] Theoretically, agents that enhance the NO-cGMP and/or cAMP signal transduction pathway such as nitrovasodilators or PDE inhibitors may prove beneficial in the treatment of erectile dysfunction. However, papaverine, a nonspecific PDE inhibitor, causes pain during injection, prolonged erections and cavernous fibrosis in a significant number of patients.[18] Recent basic research

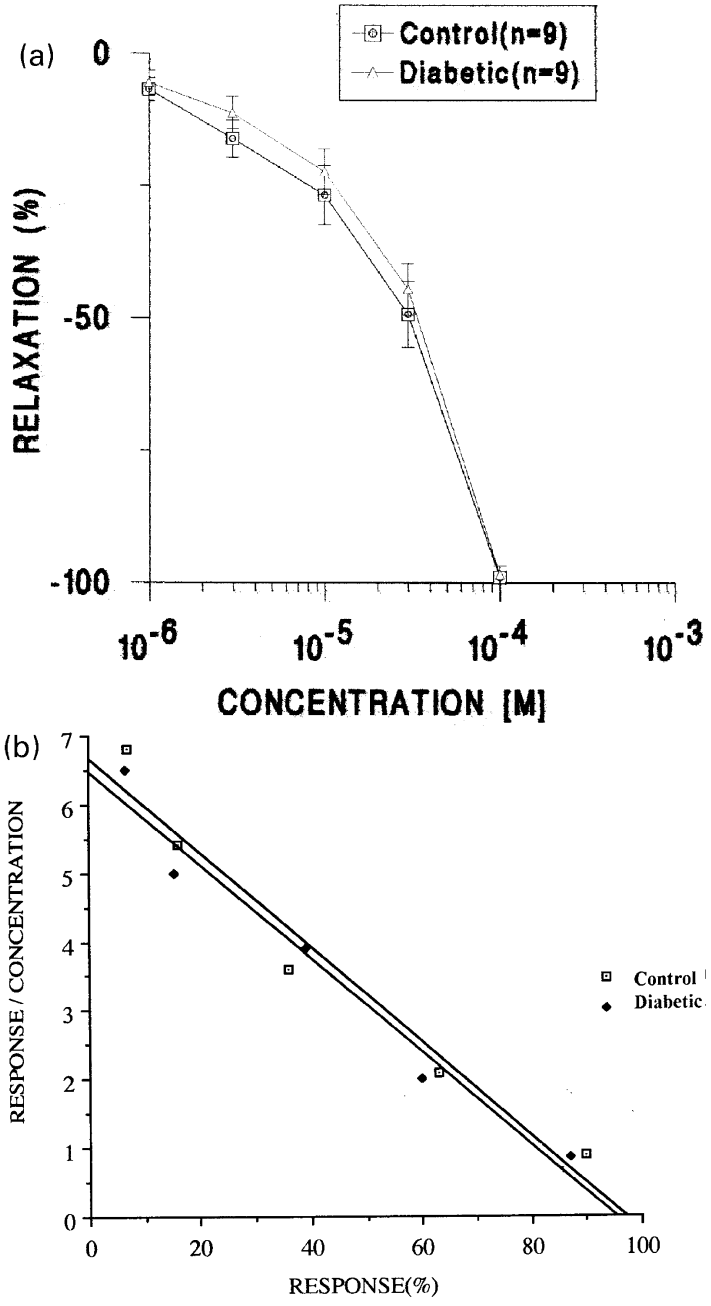


Figure 2 (a) Zaprinast concentration–response curves in isolated rabbit corpus cavernosum strips precontracted with phenylephrine $10^{-6}-3\times10^{-6}$M. Each point is expressed as a percentage of the contraction induced by phenylephrine and is given as the mean ± s.e.m. Numbers in parentheses indicate the number of preparations used. (b) Scatchard analysis. E_m% and pD_2 values shown in Table 2. ($r=0.98$ for controls and $r=0.968$ for diabetic animals).

suggests that the concept of isoenzyme selective PDE inhibition may be applicable in the treatment of erectile dysfunction.[19] Recently, a specific PDE V inhibitor sildenafil was introduced in clinical studies. In studies of human corpus cavernosum, sildenafil enhanced relaxation to electrical stimulation.[20] It has a very definite effect upon erectile activity in humans[21] and sildenafil promotes penile erection through increased intracellular cGMP in response to sexual stimulation potentiating smooth muscle relaxation.[22]

In this study, papaverine was used as a standard inhibitor, and all results obtained with other more specific inhibitors were compared with papaverine. Zaprinast and sulmazole, cGMP-specific PDE inhibitors,[23] relaxed corpus cavernosum with pD_2

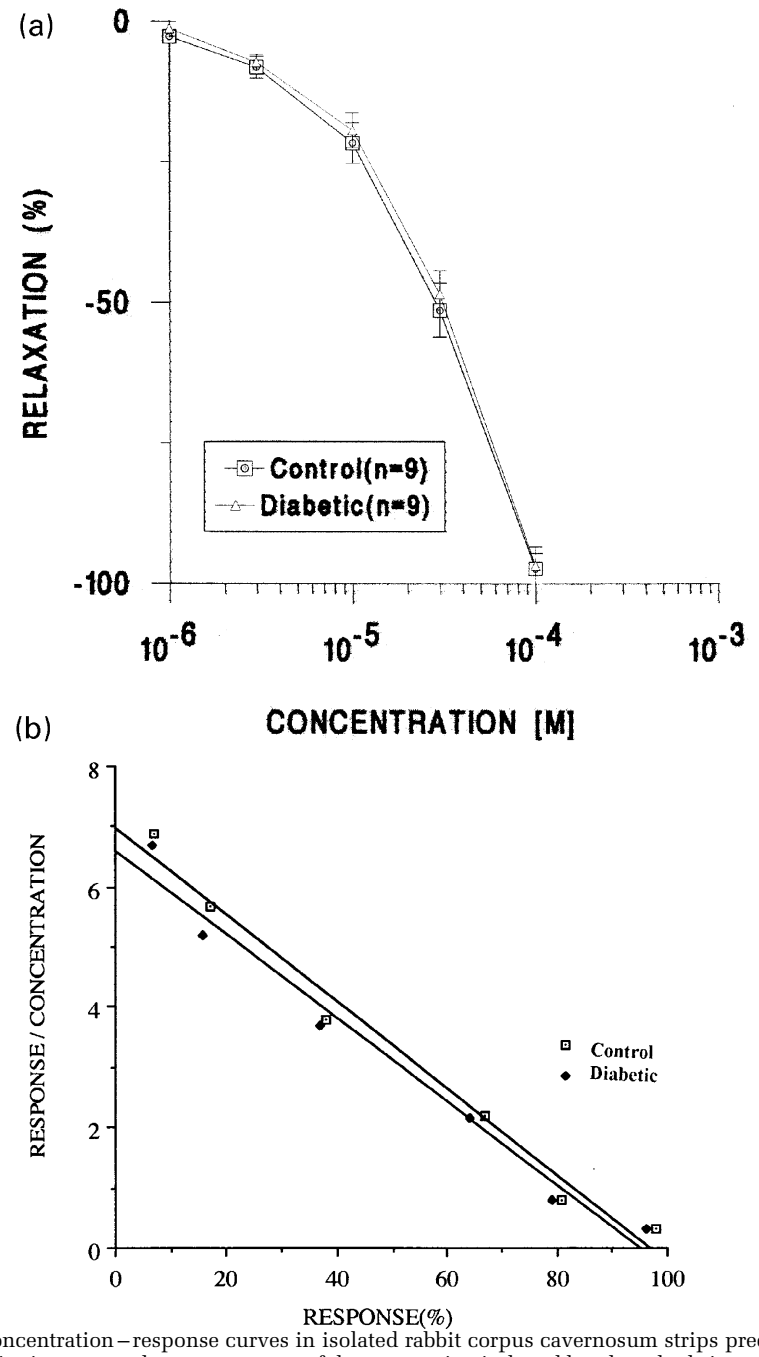


Figure 3 (a) Sulmazole concentration–response curves in isolated rabbit corpus cavernosum strips precontracted with phenylephrine $10^{-6}-3\times10^{-6}$ M. Each point is expressed as a precentage of the contraction induced by phenylephrine and is given as the mena ± s.e.m. Numbers in parentheses indicate the number of preparations used. (b) Scatchard analysis. E_m % and pD_2 values shown in Table 2. ($r=0.981$ for controls and $r=0.975$ for diabetic animals).

values similar to papaverine. In dogs, intracavernous injection of zaprinast enhanced tumescence induced by pelvic nerve stimulation[5] and in human corpus cavernosum, zaprinast enhanced relaxations caused by electric field stimulation or exogenously applied nitric oxide.[17] However, this was not confirmed in rabbit erectile tissue since pretreatment with zaprinast did not significantly augment the inhibitory effects of linsidamine (SIN-1), an NO donor.[24] In our study, amrinone, a potent cGMP-inhibited PDE inhibitor, relaxed corpus cavernosum and the pD_2 values were similar to papaverine but the E_m value was lower when compared with papaverine, sulmazole or zaprinast, thus confirming previous reports.[25]

The association of erectile dysfunction and diabetes is supported by evidence from experimental models in the rabbits.[11] Our previous studies in

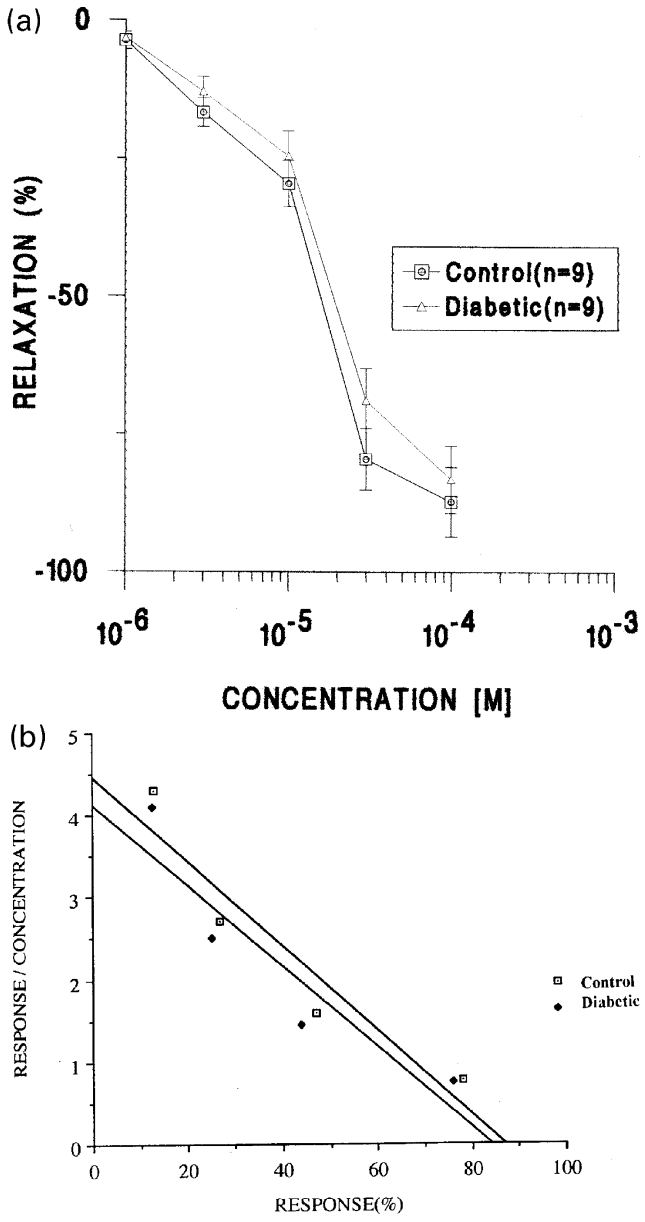


Figure 4 (a) Amrinone concentration–response curves in isolated rabbit corpus cavernosum strips precontracted with phenylephrine ($10^{-6}-3\times10^{-6}$ M. Each point is expressed as a percentage of the contraction induced by phenylephrine and is given as the mean ± s.e.m. Numbers in parentheses indicate the number of preparations used. (b) Scatchard analysis. E_m% and pD_2 values shown in Table 2. ($r=0.898$ for controls and $r=0.860$ for diabetic animals).

the blood vessels and corpus cavernosum smooth muscle show that diabetes impairs endothelium mediated and neurogenic relaxation, suggesting a possible common pathophysiological mechanism with alteration in the nitric oxide cGMP pathway.[26–28] It is reported that the impairment of endothelium-mediated relaxation of cavernosal smooth muscle seems to play a more important role in the pathogenesis of diabetogenic impotence rather than the problem of smooth muscle itself.[29]

Table 2 Maximum relaxation response (E_m % of phenylephrine-induced contraction) and pD_2 values on exposure to agonist in strips of corpus cavernosum obtained from the two groups of rabbits

	Control	n	*Diabetic*	n
Papaverine				
E_m	98.7 ± 1.3	9	96.5 ± 3.5	9
pD_2	4.57 ± 0.051	9	4.72 ± 0.063	9
Sulmazole				
E_m	98 ± 1.89	9	97 ± 2.7	9
pD_2	4.74 ± 0.037	9	4.79 ± 0.043	9
Zaprinast				
E_m	97.7 ± 2.1	9	96.9 ± 2.92	9
pD_2	4.55 ± 0.023	9	4.58 ± 0.032	9
Amrinone E_m	87 ± 7.3*	9	84 ± 5.2	9
pD_2	4.85 ± 0.087	9	4.95 ± 0.073	9

*$P < 0.05$ statistically different from papaverine, zaprinast or sulmazole.
n = number of observations.

Sullivan *et al*[30] reported that the formation of NO and PGI_2 and adenylyl cyclase activity but not guanylyl cyclase are impaired in the corpus cavernosum of diabetic rabbits, thereby suggesting that endothelial dysfunction is a major contributor to erectile dysfunction in diabetes mellitus. Although it was reported that impaired adenylate or guanylate cyclase activity does not contribute to erectile dysfunction in diabetic rats,[31] it was found recently that the hydrolysis of cAMP and cGMP was significantly reduced in both penile and aortic tissue from diabetic rats, and such a reduction of PDE activity can result in increased intracellular cyclic nucleotide levels leading to corporal smooth muscle relaxation and erection. Authors suggest that the enhanced cyclic nucleotide synthesis and decreased degradation constitute an adaptive response to the counteract effects of diabetes mellitus on erectogenic mechanisms. Consequently, the altered activity of PDE enzyme systems is not related to the pathogenesis of diabetic erectile function aetiologically.[32] In the present study, comparing the relaxant responses of the precontracted nondiabetic and diabetic rabbit cavernous tissue strips to the phosphodiesterase inhibitors *in vitro*, there was no difference between two groups.

In conclusion, our *in vitro* studies showed a concentration-related relaxant response of selective PDE-inhibitors on cavernous tissue from control and diabetic rabbits. The data presented provide the possibility of using selective PDE III and V inhibitors in the treatment of diabetic erectile dysfunction.

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